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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/699,136	Applicant(s) SANTI ET AL.
	Examiner Kathleen M Kerr	Art Unit 1652

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *29 January 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *6,7* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Application Status

1. A preliminary amendment was filed on June 15, 2001 to amend the specification. Claims 1-25 are pending in the instant application and will be examined herein.

Priority

2. The instant application is granted the benefit of priority for the U.S. Provisional Application No. 60/161,703 filed on October 27, 1999 and 60/206,082 filed on May 18, 2000 as requested in the declaration and the first lines of the specification. However, priority to U.S. Provisional Application No. 60/161,414 filed on October 25, 1999 is **NOT** granted because said application was filed more than 1 year before the filing of the instant application.

Information Disclosure Statement

3. The information disclosure statements filed on June 18, 2001 (Paper No. 6) and January 29, 2002 (Paper No. 7) have been reviewed, and their references have been considered as shown by the Examiner's initials next to each citation on the attached copy.

Drawings

4. The drawings have been approved by the Draftsmen and are, therefore, entered as formal drawings acceptable for publication upon the identification of allowable subject matter.

Compliance with the Sequence Rules

5. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). However, this application fails to **fully** comply with the requirements of 37 C.F.R. § 1.821 through 1.825; Applicants' attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990).

a) On page 67, a DNA sequence and an amino acid sequence are disclosed without benefit of SEQ ID NO.

If the noted sequences are in the sequence listing as filed, Applicants must amend the specification to identify the sequences appropriately by SEQ ID NO. If the noted sequences are not in the sequence listing as filed, Applicants must provide (1) a substitute copy of the sequence listing in both computer readable form (CRF) and paper copy, (2) an amendment directing its entry into the specification, (3) a statement that the content of the paper and CRF copies are the same and, where applicable, include no new matter as required by 37 C.F.R. § 1.821 (e) or 1.821(f) or 1.821(g) or 1.821(b) or 1.825(d), and (4) any amendment to the specification to identify the sequences appropriately by SEQ ID NO.

Objections to the Specification

6. The specification is objected to for containing incorrect priority data in the first lines of the specification. Priority has **not** been granted for 60/161,414 for the reasons cited above. Deletion of this reference is required.

7. The specification is objected to for containing figures and/or graphs embedded within the text.

a) On page 58, a figure is disclosed.

b) On page 59, a graph is disclosed.

c) On page 62, a graph is disclosed. Also, the description of this graph of page 62 includes colors that are not reflected in the graph itself.

d) On page 74, a figure is disclosed.

e) On page 82, a graph is disclosed.

f) On page 83, a graph is disclosed.

g) On page 84, a figure is disclosed.

h) On page 87, a figure is disclosed.

Appropriate correction to the specification is required.

8. In the specification, the Abstract is objected to for not completely describing the disclosed subject matter. It is noted that in many databases and in foreign countries, the Abstract is crucial in defining the disclosed subject matter, thus, its completeness is essential. The Examiner suggests the inclusion of the gene names, such as epimerase, birA (biotin transferase) and methylmalonyl CoA mutase, and the inclusion of particular kinds of cells used for completeness.

Objections to the Claims

9. Claim 8 is objected to under 37 C.F.R. § 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel

the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. The limitation of being prokaryotic or eukaryotic encompasses all possible host cells, and, thus, does not further limit the parent claim that is already drawn to all host cells.

10. Claims 14, 19, and 20 are objected to under 37 C.F.R. § 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. The phrase "derived from" does not particularly limit the source of the mutase or epimerase since any mutase or epimerase can be used an altered without limit to meet the criteria of the instant claims. See also, the 35 U.S.C. § 112, second paragraph rejection of this phrase below.

Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

11. Claims 1-5 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In Claims 1 and 2, the term "precursor required for biosynthesis of the product" is unclear as to its metes and bounds. Must the precursor be the immediate precursor of the product? Can the precursor simply be required for cell growth that facilitates all biosynthetic processes? The metes and bounds of the term are unclear.

12. Claims 2-25 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In Claim 2, the term "substantially" is a relative term that renders the claims indefinite. The term "substantially" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

13. Claim 3 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The term "primary metabolite" is unclear as to its metes and bounds. The specification focuses on secondary metabolites, particularly polyketides. Moreover, primary metabolites are typically considered to be required for cell growth so the phrase "produced in a first cell but not in a second heterologous cell" is confusing. Is the second cell an auxotroph? What does the term "heterologous" mean in this context? Must the second cell be a mutant strain of the first cell?

14. Claims 5-11 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The combination of "modular, iterative, or fungal" is confusing since fungal PKSs are iterative PKSs; the appropriate categorization of PKSs is ---modular, aromatic, or fungal--- since both aromatic and fungal are iterative PKSs.

15. Claims 5-25 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The abbreviation "PKS" is used in Claim 5 without definition upon its first occurrence. The Examiner suggests replacing "PKS" with ---polyketide synthase (PKS)--- for clarity.

16. Claims 14-17, 19, and 20 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 14, 19, and 20 contain the phrase "derived from". The metes and bounds of this phrase are unclear. Must the intended enzyme be native to the source? Or can the enzyme be native to the source and altered without limitation (except that function is retained) then used? The latter is equivalent to *any* mutase or epimerase.

17. Claims 15 and 16 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The phrase "modified to overexpress" when referring to a host cell is unclear. Typically, the terms "modify" and "overexpress" are reserved for DNA in the art. Is the genome of the host cell intended to be modified? Or can the host cells be treated with additives to facilitate the appropriate overexpression?

18. Claim 23 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The phrase "**the** *birA* gene" (emphasis added) is unclear. No particular *birA* gene is pointed to for overexpression? Is the endogenous E. coli *birA* gene intended? It is unclear.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

19. Claims 1-25 are rejected under 35 U.S.C. § 112, first paragraph, written description, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The instant claims are drawn to host cells transformed with genes that are claimed solely by function and without any structural limitations. Particularly in Claims 1 and 2, (1) the enzyme capable of making a product and (2) the enzyme capable of making a precursor required for product biosynthesis are claimed **solely** by function. In dependent claims, the enzymes in (2) are further limited to (a) methylmalonyl CoA mutase, optionally from propionibacteria, or (b) propionyl CoA carboxylase; however, these limitations also claim the product **solely by function** without any structural limitations. Also, in Claims 15, 17, 18-20, and 22, a "B12 transporter gene" (optionally from *E. coli*), a "media that facilitate B12 uptake", an epimerase (optionally from propionibacteria or *Streptomyces*), and a "biotin transferase enzyme" (optionally *birA*), respectively, are limitations that help define the product **solely by function** without any structural limitations. Claims 24 and 25 also claims the product **solely by function** using enzyme names "methylmalonyl-CoA mutase" and "epimerase".

The Court of Appeals for the Federal Circuit has recently held that a "written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as be structure, formula [or] chemical name,' of the claimed subject

matter sufficient to distinguish it from other materials." University of California v. Eli Lilly and Co., 1997 U.S. App. LEXIS 18221, at *23, quoting Fiers v. Revel, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) (bracketed material in original). To fully describe a genus of genetic material, which is a chemical compound, applicants must (1) fully describe at least one species of the claimed genus sufficient to represent said genus whereby a skilled artisan, in view of the prior art, could predict the structure of other species encompassed by the claimed genus and (2) identify the common characteristics of the claimed molecules, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these.

The following are products described and claimed by function without structural limitations in the instant claims:

a) an enzyme capable of making a product
b) an enzyme capable of making a precursor required for biosynthesis of the product
c) epimerases
d) methylmalonyl CoA mutase
e) propionyl CoA carboxylase
f) B12 transporter gene
g) media that facilitate B12 uptake
h) biotin transferase enzyme.

In the case of (a) and (b) above, no structural or functional information, except for the fact that the enzyme must catalyze a reaction (a well-known art definition of an enzyme), is offered. In the case of (a), this is *any* enzyme. The specification has not provided a representative number of species of such a wide and varied genus. In the case of (c)-(h) above, *only* functional information is required to meet the limitations of the claims; no structural limitations are used in the claims. Thus, the instant claims lack adequate written description.

20. Claims 1-25 are rejected under 35 U.S.C. § 112, first paragraph, scope of enablement, because the specification, while being enabling for particular host cells comprising vectors encoding modular PKS enzymes to make polyketides, does not reasonably provide enablement for host cells comprising vectors encoding *any* enzyme to make *any* product. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. The instant claims are drawn to such a broad and varied genus, one of skill in the art would be required to perform undue experimentation to make the instantly claimed products to the full extent of their claimed scope.

The factors to be considered in determining whether undue experimentation is required are summarized In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). The Court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (Wands, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (Wands, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the

breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

The experimentation and guidance presented in the instant specification is focused *solely* on recombinant host cells for the production of polyketides using genes encoding modular PKSs. There is no limitation in the claims wherein the "enzymes capable of making a product" are modular PKSs, although this limitation is implied in Claim 12. The specification does not enable the use of other enzymes in the production of polyketides; the Examiner notes that just because a polyketide can be synthesized by a modular PKS (limitation in Claim 12) does not limit the enzyme of Claim 1. The nature of the invention is enormously broad considering the vast array of enzymes and biosynthetic pathways known and unknown. The unpredictability of using unknown biosynthetic pathways is insurmountable for one of ordinary skill in the art without undue experimentation. Thus, the instant claims are not enabled to the full extent of their scope.

Claim Rejections - 35 U.S.C. § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

21. Claims 1-2 and 4-5 are rejected under 35 U.S.C. § 102(b) as being anticipated by **Kealey** *et al.* (IDS #6, reference #13). The instant claims are drawn to host cells comprising expression vectors encoding (1) a fungal, iterative PKS enzyme capable of producing a polyketide and (2)

an enzyme that produces a precursor for the production of the polyketide, wherein the host cells without the vectors make little, if any, precursor.

Kealey *et al.* teach the co-expression of 6-methylsalicylic acid synthase (6-MSAS) and phosphopantheteinyl transferase in *E. coli* and *S. cerevisiae* host cells; 6-methylsalicylic acid (6-MSA) is a polyketide and 6-MSAS is a fungal, iterative PKS (see Abstract and page 505, left column). In terms of the instant claims, 6-MSAS is an enzyme capable of making a polyketide and phosphopantheteinyl transferase is an enzyme required to make a precursor required for the biosynthesis of the polyketide, that precursor being the functional holo-6-MSAS. In *E. coli,* or *S. cerevisiae*, holo-6-MSAS is not effectively produced, although small amounts may be made, without the expression of the phosphopantheteinyl transferase. Both *E. coli* and *S. cerevisiae* lack a natural biosynthetic pathway for the production of 6-MSA.

22. Claims 2-3 are rejected under 35 U.S.C. § 102(b) as being anticipated by **Tuchman** *et al.* (Enhanced Production of Arginine and Urea by Genetically Engineered *Escherichia coli* K-12 Strains. Applied and Environmental Microbiology (1997) 63(1):33-38). The instant claims are drawn to host cells comprising expression vectors encoding (1) an enzyme capable of producing a product and (2) an enzyme that produces a primary metabolite that is a precursor required for the synthesis of the product, wherein the host cells without the vectors make relatively little precursor.

Tuchman *et al.* teach the overexpression of *carAB*, *argI*, and *rocF* genes in *E. coli* for the production of citrulline and ultimately arginine and urea (see Abstract and Figure 1). The *carAB* gene product, carbamyl phosphate synthetase, produces carbamoyl phosphate (a primary

metabolite); carbamoyl phosphate is a precursor of citrulline, which reaction is catalyzed by the *argI* gene product (see Figure 1).

Claim Rejections - 35 U.S.C. § 103

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

23. Claims 1-2 and 4-6 are rejected under 35 U.S.C. § 103(a) as being unpatentable over **Stassi** *et al.* (IDS #7, reference #11) in view of **Kao** *et al.* (IDS #7, reference #6). Claim 6, encompassing all the limitations of Claims 1, 2, 4, and 5, is drawn to recombinant host cells comprising expression vectors that encode (1) an enzyme that produces ethylmalonyl-CoA and (2) a modular PKS enzyme that uses ethylmalonyl-CoA to produce a polyketide product.

Stassi *et al.* teach *S. erythraea* host cells comprising a modified genomic copy of the erythromycin PKS gene cluster; while the modified erythromycin PKS is expressed, detectable amounts of polyketides are not produced. Stassi *et al.* further teach that, for the modified erythromycin PKS to produce polyketides, said host cells must also contain appropriate precursors for the enzyme reactions of the modified erythromycin PKS – one of these precursors being ethylmalonyl-CoA. To introduce said precursor, Stassi *et al.* further teach said host cells transformed with an expression vector comprising a gene encoding crotonyl-CoA reductase which reductase produces ethylmalonyl-CoA (see Abstract and page 7308). Stassi *et al.* teach that the expression of crotonyl-CoA reductase is necessary for the production of the

erythromycin derivative from the modified erythromycin PKS because of the alteration in the endogenous erythromycin PKS genes in the *S. erythraea* host cells wherein the acyltransferase (AT) domain of module 4, which is methylmalonyl-specific, is replaced with the AT domain of module of 5 the niddamycin PKS which is ethylmalonyl-specific (see page 7308), which replacement AT domain requires ethylmalonyl-CoA as a precursor.

While Stassi *et al.* teach the requirement for supplying enzymes (via their encoding genes on expression vectors) that can produce appropriate precursors for recombinant erythromycin PKS enzymes, Stassi *et al.* do not teach expression of **both** the recombinant erythromycin PKS and the precursor-producing enzyme **from vectors** in a heterologous host cell since, in Stassi *et al.*, the erythromycin PKS genes are altered on the endogenous chromosome.

Kao *et al.* teach the heterologous expression of the complete erythromycin PKS system, which is a modular PKS system, in modified, recombinant *S. coelicolor* host cells unable to produce erythromycin naturally.

It would have been obvious to one of ordinary skill in the art to make the claimed recombinant host cells containing vector-bound (1) altered erythromycin PKS genes and (2) a recombinant gene encoding crotonyl-CoA reductase (which produces the ethylmalonyl-CoA precursor) by combining the teachings of Stassi *et al.* and Kao *et al.* because Kao *et al.* teach that heterologous expression of PKS gene clusters from vectors facilitates "rapid structural manipulation" (see Abstract) and Stassi *et al.* manipulate the endogenous erythromycin PKS using more complex techniques. Thus, the alteration method of Kao *et al.*, namely using altered PKS genes on vectors, would have been an obvious, simpler variation of the method used by Stassi *et al.*, namely altering the endogenous erythromycin PKS genes. Moreover, the host cells

of the instant claims are obvious because Stassi *et al.* teach that alteration of erythromycin PKS genes in host cells, endogenously or in the obvious variation of vectors, may also require additional enzymes (added to the host cells via expression vectors containing the encoding genes) to supply appropriate precursors in quantities sufficient for efficient erythromycin-derivative polyketide production wherein the altered erythromycin PKS enzymes now require different precursors.

One would have been motivated to produce the host cells of Stassi *et al.* with both the precursor gene and the altered erythromycin PKS genes on vectors because the manipulation of vector-bound genes, as taught by Kao *et al.*, is less technically demanding than the manipulation of genes in the host cell's genome, as taught by Stassi *et al.* Manipulation of PKS genes, particularly erythromycin PKS genes, for the production of new polyketides is well-known in the art for the purpose of production of novel, potential therapeutics.

One would have had a reasonable expectation of success that expressing both precursor-producing enzymes and altered erythromycin PKS enzymes in recombinant *S. coelicolor* host cells would effectively supply appropriate precursors for the altered erythromycin PKS enzymes because Kao *et al.* and Stassi *et al.* independently demonstrate their recombinant expression in similar (*Saccharomyces* and *Streptomyces*) host cells.

24. Claims 7-8 are rejected under 35 U.S.C. § 103(a) as being unpatentable over **Stassi** *et al.* (IDS #7, reference #11) and **Birch** *et al.* (IDS # 6, reference #9) in view of **Kao** *et al.* (IDS #7, reference #6). The instant claims are drawn to recombinant host cells comprising vectors that

encode (1) an enzyme that produces a precursor, methylmalonyl-CoA, and (2) a modular PKS that produces a polyketide product.

Stassi *et al.* teach as described above. Briefly, Stassi *et al.* teach the expression of an enzyme (crotonyl-CoA reductase) that produces ethylmalonyl-CoA in conjunction with expression of an endogenous, modified erythromycin PKS that utilizes ethylmalonyl-CoA as a precursor to erythromycin-derivative polyketide production. Particularly, Stassi *et al.* teach that appropriate precursor supply is *necessary* for polyketide biosynthesis.

Birch *et al.* teach the expression of *S. cinnamonensis* methylmalonyl-CoA mutase, which enzyme produces methylmalonyl-CoA. Birch *et al.* also teach that precursor supply is *necessary* and *can be limiting* in polyketide biosynthesis (see Abstract and page 3511, left column).

While Stassi *et al.* and Birch *et al.* each independently teach the requirement for supplying enzymes (via their encoding genes on expression vectors) that produce appropriate precursors for recombinant PKS enzymes, neither references teaches expression of **both** the PKS and the precursor-producing enzyme from vectors in a heterologous host cell.

Kao *et al.* teach the heterologous expression of the complete erythromycin PKS system, which is a modular PKS system, in modified recombinant *S. coelicolor* host cells unable to produce erythromycin naturally.

It would have been obvious to one of ordinary skill in the art to make the claimed host cells containing (1) recombinant erythromycin PKS genes and (2) a gene encoding methylmalonyl-CoA mutase by combining the teachings of Stassi *et al.*, Birch *et al.*, and Kao *et al.*, because Kao *et al.* teach that heterologous expression of PKS gene clusters from vectors facilitates "rapid structural manipulation" (see Abstract) and the ability of PKS genes to produce

combinatorial libraries of polyketide products by the recombinant alteration of said genes is well-known in the art. Moreover, it would have been obvious to include a recombinant gene encoding methylmalonyl-CoA mutase in the heterologous host cell expression systems of Kao *et al.* because both Stassi *et al.* and Birch *et al.* emphasize the need for appropriate precursor production and/or supply in the efficient biosynthesis of polyketides whose precursor requirements are problematic due to the iterative process of biosynthesis from two-carbon units. It is well-known in the art that erythromycin utilizes many methylmalonyl-CoA units per erythromycin -derivative produced.

One would have been motivated to combine the above teachings because both Stassi *et al.* and Birch *et al.* teach the necessity of sufficient amounts of precursors in heterologous host cells producing polyketides via PKS enzymes for efficient polyketide production. Manipulation of PKS genes, particularly erythromycin PKS genes, for the production of new polyketides is well-known in the art for the production of novel, potential therapeutics. Efficient production of said potential therapeutics is desirable.

One would have had a reasonable expectation of success that expressing both precursor-producing enzymes and PKS enzymes in recombinant *S. coelicolor* host cells would effectively supply appropriate precursors for the PKS enzymes because Kao *et al.*, Stassi *et al.*, and Birch *et al.* each independently demonstrate recombinant expression of the different enzymes.

25. Claims 1-2 and 4-13 are rejected under 35 U.S.C. § 103(a) as being unpatentable over **Stassi** *et al.* (IDS #7, reference #11) and **Birch** *et al.* (IDS # 6, reference #9) in view of **Barr** *et al.* (IDS # 6, reference #3). The instant claims are drawn to *E. coli*, yeast, or plant host cells

comprising vectors that encode (1) a methylmalonyl-CoA mutase and (2) a modular PKS that produces a polyketide product.

Stassi *et al.* and Birch *et al.* teach as described above. Briefly, Stassi *et al.* and Birch *et al.* teach the *necessity* of supplying sufficient amounts of polyketide precursors in recombinant host cells that express PKS genes for efficient polyketide biosynthesis in said host cells.

While Stassi *et al.* and Birch *et al.* each independently teach the requirement for supplying enzymes (via their encoding genes on expression vectors) that can produce appropriate precursors for recombinant PKS enzymes, neither reference teaches expression of **both** the PKS and the precursor-producing enzyme from vectors in a recombinant host cell.

Barr *et al.* teach recombinant methods of polyketide library production using PKS genes on expression vectors in heterologous hosts, namely *E. coli*, yeasts, and plants (see Abstract and column 6, line 65).

It would have been obvious to one of ordinary skill in the art to make the claimed recombinant host cells containing (1) recombinant PKS genes and (2) a recombinant gene encoding methylmalonyl-CoA mutase by combining the teachings of Stassi *et al.* and Birch *et al.* with Barr *et al.* because Barr *et al.* teach the ease of these heterologous hosts with the benefits of gene manipulation in the production of polyketide libraries. Moreover, it would have been obvious to include a recombinant gene encoding methylmalonyl-CoA mutase in the heterologous host cell expression systems of Barr *et al.* because both Stassi *et al.* and Birch *et al.* emphasize the need for appropriate precursor production and/or supply in the efficient biosynthesis of polyketides whose precursor requirements are problematic due to the iterative process of biosynthesis from two-carbon units.

One would have been motivated to combine the above teachings because both Stassi *et al.* and Birch *et al.* teach the necessity of sufficient amounts of precursors being produced in heterologous host cells efficiently producing polyketides via PKS enzymes. Moreover, one would have been motivated to combine the teachings because Stassi *et al.* teach that alteration in PKS genes, wherein the host cells containing said genes now require different precursors, may also require additional enzymes to supply said precursors in quantities sufficient for efficient polyketide production. Efficient production of said potential therapeutics is desirable.

One would have had a reasonable expectation of success that expressing both precursor-producing enzymes and PKS enzymes in *E. coli*, yeast, and plant host cells would effectively supply appropriate precursors for the PKS enzymes because Stassi *et al.*, Birch *et al.*, and Barr *et al.* each independently demonstrate recombinant expression of the different enzymes.

26. Claims 14-17 are rejected under 35 U.S.C. § 103(a) as being unpatentable over **Stassi** *et al.* (IDS #7, reference #11) and **Birch** *et al.* (IDS # 6, reference #9) in view of **Barr** *et al.* (IDS # 6, reference #3) and in further view of **McKie** *et al.* (IDS #7, reference #8). The instant claims are drawn to *E. coli* host cells comprising vectors that (1) encode a methylmalonyl-CoA mutase derived from propionibacteria, (2) encode a modular PKS that produces a polyketide product, and either (3a) encode an *E. coli* B12 transporter or (3b) are grown in media that facilitates B12 uptake in *E. coli*.

Stassi *et al.*, Birch *et al.*, and Barr *et al.* teach as described above. Birch *et al.* further equate the methylmalonyl-CoA mutase from *S. cinnamonensis* to said mutase from *Propionibacterium shermanii* (see Abstract). Barr *et al.* further teach the particular usefulness of

E. coli host cells due to their ease of manipulation and growth for large-scale polyketide production (see columns 6-7).

The additional reference, McKie *et al.*, teaches a particular methylmalonyl-CoA mutase from *Propionibacterium shermanii* and its efficient expression in *E. coli* host cells. McKie *et al.* also teach that methylmalonyl-CoA mutase is B12 dependent (see page 293, left column).

The addition of McKie *et al.* to the references of the 103(a) rejection of Claims 1-2 and 4-13 is obvious because Birch *et al.* specifically equate the two mutases (see Abstract). Since Stassi *et al.* teach the addition of precursor-producing enzymes (via their encoding genes on expression vectors), ***any*** enzyme having the ability to produce methylmalonyl-CoA is an obvious variation. Moreover, the additional overexpression of an *E. coli* B12 transporter gene is an obvious one since the host cells are *E. coli* host cells and the mutase requires B12. Similarly, the B12 media is also an obvious variation.

One would have been motivated to utilize the *P. shermanii* mutase because an effective expression system for said mutase in *E. coli* had already been developed (see McKie *et al.*, page 293, right column). Using said expression system, one would have been motivated to additionally use an *E. coli* B12 transporter gene and/or *E. coli* B12 media for the purpose of optimizing the activity of the mutase to produce the precursor, methylmalonyl-CoA, most productively since optimization of expressed enzyme activity is well-known in the art. The overall motivation is efficient production of polyketides in heterologous host cell fermentations; efficient production of said potential therapeutics is desirable.

One would have had a reasonable expectation of success that the *P. shermanii* mutase would have been well overexpressed in *E. coli* and highly functional with the addition of B12 augmentation due to the efficient expression system taught by McKie *et al.*

27. Claims 18 and 24-25 are rejected under 35 U.S.C. § 103(a) as being unpatentable over **Stassi** *et al.* (IDS #7, reference #11) and **Birch** *et al.* (IDS # 6, reference #9) in view of **Donadio** *et al.* (IDS # 7, reference #5) and in view of **Barr** *et al.* (IDS # 6, reference #3). The instant claims are drawn to *E. coli* or yeast host cells comprising vectors that (1) encode a methylmalonyl-CoA mutase, (2) encode a modular PKS that produces a polyketide product, and (3) encode an epimerase.

Stassi *et al.* and Birch *et al.* teach as described above. Briefly, Stassi *et al.* and Birch *et al.* teach the importance of supplying sufficient amount of polyketide precursors in host cells that express PKS genes so that said host cells efficiently produce polyketides. Birch *et al.* additionally teach the usefulness of methylmalonyl-CoA epimerase for the production of (S)-methylmalonyl-CoA (see Figure 1).

Donadio *et al.* teach that particularly the (S) enantiomer of methylmalonyl-CoA is recognized by the erythromycin PKS (see page 977, right column).

While Stassi *et al.* and Birch *et al.* each independently teach the requirement for supplying enzymes (via their encoding genes on expression vectors) that can produce appropriate precursors for recombinant PKS enzymes, neither reference teaches expression of **both** the PKS and the precursor-producing enzyme(s) from vectors in a host cell.

Barr *et al.* teach recombinant methods of polyketide library production using PKS genes on expression vectors in heterologous hosts, namely *E. coli* and yeasts (see Abstract and column 6, line 65).

It would have been obvious to one of ordinary skill in the art to make the claimed host cells containing (1) recombinant, modified PKS genes, (2) a recombinant gene encoding methylmalonyl-CoA mutase, and (3) a recombinant gene encoding a methylmalonyl-CoA epimerase by combining the teachings of Stassi *et al.*, Birch *et al.*, and Donadio *et al.* with Barr *et al.* because Barr *et al.* teach the ease of these heterologous hosts and the benefits of gene manipulation in the production of polyketide libraries and Stassi *et al.* and Birch *et al.* point out the need to have sufficient amounts of precursors in the polyketide-producing host cells and the combination of Birch *et al.* and Donadio *et al.* demonstrate the requirement for both the mutase and the epimerase to make an appropriate PKS precursor, (S)-methylmalonyl-CoA.

One would have been motivated to combine the above teachings because both Stassi *et al.* and Birch *et al.* teach the necessity of sufficient amounts of precursors being produced in heterologous host cells efficiently producing polyketides via PKS enzymes. Moreover, one would have been motivated to combine the teachings because Stassi *et al.* teach that alteration in PKS genes, wherein the host cells containing said genes now require different precursors, may also require additional enzymes to supply said precursors in quantities sufficient for efficient polyketide production.

One would have had a reasonable expectation of success that expressing both precursor-producing enzymes and PKS enzymes in *E. coli*, yeast, and plant host cells would effectively

supply appropriate precursors for the PKS enzymes because Stassi *et al.*, Birch *et al.*, and Barr *et al.* each independently demonstrate recombinant expression of the different enzymes.

28. Claim 21 is rejected under 35 U.S.C. § 103(a) as being unpatentable over **Stassi** *et al.* (IDS #7, reference #11) and **Donadio** *et al.* (IDS # 7, reference #5) in view of **Barr** *et al.* (IDS # 6, reference #3). The instant claims are drawn to *E. coli* host cells comprising vectors that encode (1) a propionyl-CoA carboxylase and (2) a modular PKS that produces a polyketide product.

Stassi *et al.* teach as described above. Briefly, Stassi *et al.* teach the importance of supplying sufficient amounts of polyketide precursors in host cells that express PKS genes for efficient polyketide biosynthesis in said host cells.

Donadio *et al.* teach as described above. Donadio *et al.* also teach that (S)-methylmalonyl-CoA can be produced by propionyl-CoA carboxylase.

While Stassi *et al.* teach the requirement for supplying enzymes (via their encoding genes on expression vectors) that can produce appropriate precursors for recombinant PKS enzymes, neither reference teaches expression of **both** the PKS and the precursor-producing enzyme from vectors in a host cell.

Barr *et al.* teach recombinant methods of polyketide library production using PKS genes on expression vectors in heterologous hosts, namely *E. coli* (see Abstract and column 6, line 65).

It would have been obvious to one of ordinary skill in the art to make the claimed host cells containing (1) recombinant PKS genes and (2) a recombinant gene encoding propionyl-CoA carboxylase by combining the teachings of Stassi *et al.* and Donadio *et al.* with Barr *et al.*

because Barr *et al.* teach the ease of these heterologous hosts with the benefits of gene manipulation in the production of polyketide libraries. Moreover, it would have been obvious to include a recombinant gene encoding propionyl-CoA carboxylase in the heterologous host cell expression systems of Barr *et al.* because Stassi *et al.* emphasize the need for appropriate precursor production and/or supply in the efficient biosynthesis of polyketides whose precursor requirements are problematic due to the iterative process of biosynthesis from two-carbon units.

One would have been motivated to combine the above teachings because Stassi *et al.* teach the necessity of sufficient amounts of precursors being produced in heterologous host cells efficiently producing polyketides via PKS enzymes. Moreover, one would have been motivated to combine the teachings because Stassi *et al.* teach that alteration in PKS genes, wherein the host cells containing said genes now require different precursors, may also require additional enzymes to supply said precursors in quantities sufficient for efficient polyketide production. One would have had a reasonable expectation of success that expressing both precursor-producing enzymes and PKS enzymes in *E. coli* host cells would effectively supply appropriate precursors for the PKS enzymes because Stassi *et al.*, Donadio *et al.*, and Barr *et al.* each independently demonstrate recombinant expression of the different enzymes.

Additionally Cited Prior Art

29. The following references have not been relied upon for art rejections above, but are considered pertinent to the subject matter of the instant application and the elected invention:

a) Betlach *et al.* USPN 6,262,340 B1published July 17, 2001 with an effective filing date of July 10, 1997. Betlach *et al.* teach polyketide synthesis using recombinant PKS gene clusters in plants and the importance of precursor availability in such systems.

b) Julien *et al.* USPN 6,303,342 B1 published October 16, 2001 with an effective filing date of November 20, 1998. Julien *et al.* teach enhancement methods of polyketide production in host cells including increasing expression rates and increasing precursor supplies.

c) Hunaiti *et al.* Source of Methylmalonyl-Coenzyme A for Erythromycin Synthesis: Methylmalonyl-Coenzyme A Mutase from *Streptomyces erythreus*. Antimicrobial Agents and Chemotherapy (1984) 25(2):173-178. Hunaiti *et al.* teach the *S. erythreus* produces erythromycins from methylmalonyl-CoA produced by methylmalonyl-CoA mutase, a B12-dependent enzyme.

d) Wawszkiewicz *et al.* Propionyl-CoA* Dependent $H^{14}CO_3^-$ Exchange into Methylmalonyl-CoA in Extracts of *Streptomyces erythraeus*. Biochemische Zeitschrift (1964) 340:213-227. Wawszkiewicz *et al.* teach that erythronolide production involves methylmalonyl-CoA and propionyl-CoA.

e) Gokhale *et al.* Dissecting and Exploiting Intermodular Communication in Polyketide Synthases. Science (1999) 284:482-485. Gokhale *et al.* teach methods of PKS gene expression and methods of making polyketides in heterologous *E. coli* host cells.

Conclusion

30. Claims 1-25 are not allowed for the reasons identified in the numbered sections of this Office action. Applicants must respond to the objections/rejections in each of the numbered sections in this Office action to be fully responsive in prosecution.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kathleen M Kerr whose telephone number is (703) 305-1229. The examiner can normally be reached on Monday through Friday, from 8:30am to 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathupura Achutamurthy can be reached on (703) 308-3804. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-0294 for regular communications and (703) 305-3014 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

KMK
February 13, 2002